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NO. 1131

HORMONAL CONSEQUENCES OF ORGANOPHOSPHATE POISONING (U)[1]

by

J.G. Clement

Project No. 13D20

December 1985

[1] Originally presented at a Symposium on the Prophylaxis and Therapy of Chemical Agent Poisoning, 22 – 24 April 1985, Swedish Medical Facility, Stockholm, Sweden

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ABSTRACT

Hormonal changes were estimated after poisoning with an extremely toxic organophosphate, soman (pinacolyl methylphosphonofluoridate). Soman produced a significant ($p<0.05$) increase in serum corticosterone, thyroxine and triiodothyronine concentrations at 3, 6 and 9 hrs after poisoning. However, after 22 hrs the levels were not significantly different from control. Plasma ACTH levels were decreased significantly ($p<0.05$) 3 hrs after soman at 100 μg/kg but not after 267 μg/kg. Serum testosterone levels were decreased significantly ($p<0.01$) 6 and 9 hrs after soman poisoning but had returned to control levels by 22 hrs.

Soman (100 μg/kg) produced an immunosuppressive response when

administered at 24 hrs after an antigen (sheep red blood cells). However a similar response was obtained in adrenalectomized mice suggesting that some mechanism other than elevated corticosterone was responsible for the immunosuppressive effect.

Soman poisoning produced an intense hypothermia. It is possible that the hormonal changes noted after soman poisoning are due to a reduction in metabolism and excretion, a result of the hypothermia and not a direct action of soman.

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INTRODUCTION

In recent years there has been a resurgence in the research effort, not only in various military sponsored research laboratories but also in universities and private research companies, devoted to the mechanism and treatment of organophosphate poisoning, particularly with regards to the so called "nerve gases". During the past four decades research has tended to concentrate on the direct cholinergic effects of nerve agent poisoning. Only recently, within the last decade, have researchers started to investigate seriously non-cholinergic effects of organophosphate poisoning (Table 1). In addition, organophosphates interact with a variety of other enzymes besides acetylcholinesterase (Table 2). In most cases, the significance of these interactions with other enzymes in the etiology of organophosphate poisoning

remains to be determined.

The significance of the studies in Tables 1 and 2 become very relevant in light of the development of new and more effective treatment regimens such as pyridostigmine prophylaxis (Gall, 1981; Gordon et al., 1978) and bispyridinium oximes such as HI-6, HGG-12, HGG-42 and BDB-27 (Clement, 1983a; Clement, 1981; Maksimovic et al., 1980) combined with a standard anticholinergic such as atropine.

In a chemical warfare scenario employing nerve gases, the use of these more effective prophylactic and therapeutic measures combined with personal protective equipment would make it highly probable that more people would survive such an attack. With this in mind the emphasis has been shifting to the examination of physiological and/or biochemical changes which might occur in a survivor of nerve agent poisoning concentrating on the nerve agent soman (pinacolyl methylphosphonopfluoridate).

It was recognized early that hormones modulated organophosphate toxicity. Pretreatment of rodents with various steroids has been found to reduce the toxicity of various organophosphosphates (Robinson et al., 1978; Selye, 1970; Robinson et al., 1976; Dubois et al., 1949; Swann et al., 1958). Administration of diethylstilbestrol to male rats increased the toxicity of parathion while testosterone administration decreased the toxicity of parathion to female rats (Dubois et al., 1949; Swann et al., 1958). Selye (1970) coined the term "catatoxic" (from the Greek word kata which means down, against) to describe steroids exhibiting this activity. In these particular cases it was presumed that the protective effect against the toxicity of parathion was due to induction of drug metabolizing enzymes which reduced the toxicity through an enhanced metabolic breakdown (Robinson et al, 1978). These studies demonstrated that certain steroids possessed other biochemical/physiological actions which can attenuate organophosphate

toxicity. Other non-steroidal inducers of hepatic microsomal enzymes (e.g. phenobarbital) reduced the toxicity of a number of organophosphates (Dubois and Kinoshita, 1968) probably through the same mechanism, namely an enhanced in vivo metabolism. However, in the case of soman, induction of hepatic microsomal enzymes does not explain the decreased toxicity following phenobarbital treatment since soman was not a substrate for the drug metabolizing enzyme system in the liver. In addition, phenobarbital pretreatment did not increase the activity of somanase in mice (Clement, 1983b). The protective effect provided by phenobarbital against soman toxicity was the result of an increased concentration of plasma aliesterase, an enzyme which binds and thus detoxifies soman (Clement, 1983b).

Hormonal changes can produce a myriad of effects in vivo which, if not considered, may result in incorrect interpretations of the biochemical and/or neurochemical changes which occur after organophosphate poisoning. Are the changes one is examining a primary effect of nerve agent poisoning or are they a secondary effect due to some hormonal imbalance? The results presented in this paper illustrate the effects of nerve agent poisoning on hormones such as corticosterone, adrenocorticotrophic hormone (ACTH), thyroxine, triiodothyronine and testosterone, an area which to this point has been been largely ignored.

RESULTS AND DISCUSSION

Effect of Nerve Agent Poisoning on the Adrenal-Pituitary Axis

Glucocorticoids are extremely important hormones regulating or modulating a wide variety of biochemical and/or physiological processes in vivo (Table 3). In rodents the primary glucocorticoid produced by the

adrenal gland is corticosterone due to the lack to the enzyme 17-hydroxylase. Previous investigators reported that anticholinesterase poisoning was similar to a generalized stress reaction, both situations in which the plasma corticosterone concentration was elevated (Ray and Poddar, 1983; Szot and Murphy, 1970; Murphy, 1969; Szot and Murphy, 1971; Polakovic et al., 1984). However, this effect was not common to all anticholinestease compounds. Civen et al. (1977a) found that dichlorvos depressed plasma corticosterone concentrations probably by inhibition of adrenal steroidogenesis (Civen et al., 1977b).

The effect of soman poisoning on plasma corticosterone levels has been investigated at DRES. Mice, housed 5/cage, were injected with either saline, soman (100 μg/kg, sc) or HI-6 (50 mg/kg; ip) and atropine (17.4 mg/kg; ip) 5 min before soman (287 μg/kg; sc) at 1000 hrs. At various times of the day (1000, 1300, 1600 and 1900 hrs) mice were sacrificed by decapitation; a method having the least effect on corticosterone concentrations (Carney and Walker, 1973). Blood was collected in Microtainer® tubes (Becton-Dickenson) and centrifuged, after clot formation, to separate the serum. Samples were then frozen and stored at -20°C until analysis by RIA.

Serum corticosterone concentrations measured by RIA (Endocrin Sci, Tarzana, Ca) was elevated significantly within 3 hrs of soman (100 and 287 μg/kg) administration (Table 4). By 1900 hrs the corticosterone levels following 100 μg/kg soman had returned to control levels whereas those following 287 μg/kg soman were still significantly ($p<0.01$) elevated. The serum corticosterone concentrations measured at 0800 and 1600 hrs the day following soman (100 and 287 μg/kg) administration were not significantly different from the control group (data not shown).

ACTH, secreted by the anterior pituitary, stimulates the production and thus subsequent release of corticosterone by the adrenal cortex. The

plasma concentration of corticosterone is regulated by negative feedback inhibition either at the level of the hypothalmus or anterior pituitary. Plasma ACTH levels measured by RIA (Immuno Nuclear Corp., Stillwater, Min.) following soman (100 μg/kg) were significantly different ($p<0.05$) at 1300 hrs (Table 5) but returned to control levels by 1900 hrs whereas ACTH levels following soman (287 μg/kg) were not significantly different from control at any of the time periods examined even though serum corticosterone concentrations were elevated. This lack of negative feedback inhibition may be due to the presence of atropine which has been shown to interfere with ACTH secretion (Hedge and Smelik, 1968).

The corticosterone concentrations increased in the absence of an enhanced ACTH secretion. This could result from a decreased steroid metabolism by the liver, the main site of steroid metabolism (Berliner and Dougherty, 1961) and/or a direct stimulatory effect of soman on the adrenal cortex. ACTH stimulates an increase in c-AMP in the adrenal cortex which then increases phosphorylase activity and ultimately the production and output of corticosterone. Sevaljevic et al. (1984) reported that soman poisoning stimulated adenylate cyclase activity and increased the c-AMP content in synaptosomes following poisoning by 1.3 LD_{50} soman. If the same effect occured in the adrenal gland it may be responsible for a direct action. Recently it was reported that physostigmine increased plasma corticosterone in hypophysectomized rats which indicated a direct effect on the adrenal gland (Kolta and Soliman, 1981). Alternatively, an effect of soman on metabolism of corticosterone is a possibility. Following soman (100 and 287 μg/kg) poisoning the activities of mixed hepatic function oxidases such as aniline hydroxylase and p-nitroanisole O-demethylase were not inhibited whereas the activity of ethylmorphine N-demethylase was enhanced (Table 6) suggesting that impairment of liver metabolism was not a general feature following poisoning. However a specific effect on hormone metabolism cannot be dismissed (Donovan et al., 1978). Following soman administration

mice became profoundly hypothermic (Fig 1). This intense hypothermia undoubtedly decreased the overall metabolic rate in vivo. This, combined with a decrease in cardiac output and blood flow to the liver (Ballard, 1974), could contribute to the increase in serum corticosterone. Further to this argument other investigators have concluded that plasma corticosterone changes were not related to brain cholinesterase inhibition and thus was not due to a cholinergic mechanism (Szot and Murphy, 1970; Szot and Murphy, 1971; Osicka-Koprowska et al., 1984; Ray and Poddar, 1983).

Immunosuppressive Effects of Nerve Agent Poisoning

Various laboratories have reported that anticholinesterases possess certain immunotoxic properties (Vos and Krajnc, 1983; Casale et al., 1984, Casale et al., 1983; Becker, 1975; Street, 1981; Pipy et al., 1982). Since glucocorticoids have immunosuppressive actions, further experiments were conducted examining the effect of soman poisoning on the immune system. Initially mice were injected with sheep red blood cells (SRBC) then injected at various times thereafter with soman. Four days after SRBC injection the mice were sacrificed, the spleen was removed and prepared for a standard plaque forming assay (Jerne et al., 1963). Soman produced the greatest immunosuppressive effect when administered 24 hrs after the SRBC (Fig 2). It interfered with the early stages of the immune response but was virtually ineffective if administered 72 hrs post antigen challenge. Soman (50 μg/kg; sc) administered 24 hrs after SRBC was not immunosuppressive whereas soman [287 μg/kg, sc; pretreated 5 min before with atropine (17.4 mg/kg, ip) plus HI-6 (50 mg/kg, ip)] produced the same degree of immunosuppression as that found after soman (100 μg/kg; sc). Additional experiments with equitoxic doses (0.75 LD_{50}) of sarin (130 μg/kg) and tabun (200 μg/kg) demonstrated that these were also immunosuppressive. However they both were less potent than soman producing 54% and 37% suppression of the plaque response, respectively.

The time course of the immunosuppressive effect of soman indicated that interference at a certain point in the immune response during the rapid proliferation phase, probably when a certain esterase has been activated (Becker, 1975), was all that was required to produce the effect. Administration of soman early or late in the immune response was without effect. Makinadam et al. (1970) stated that for the immunosuppressive action of adrenal steroids to be manifested "...all that is needed is an hour of damage at a crucial time in the evolution of the antibody response." The elevated steroid levels do not have to be maintained because corticosterone will produce effects which persist long after the hormone has disappeared or decreased below the initial threshold level (Berliner and Dougherty, 1961).

In vitro cytotoxicity tests to spleen and thymus cells revealed no direct toxicity to these cells at doses of 50 μg/ml of soman, sarin or tabun. The concentrations are well above those producing toxicity in vivo. The compounds also failed to inhibit polymorphonuclear phagocytosis.

The above results suggest that soman poisoning may attenuate the animal's response to a bacterial infection. In the next series of experiments the effect of soman poisoning on the toxicity of an infectious agent such as Neisseria meningitidis was examined. As the toxicity following N. meningitidis is the result of an immunological response to the toxoid produced by this bacteria (DiNinno, V., personal communciation), any compromise of the immune system should be reflected in lower toxicity. The results in Table 7 support this hypothesis. Iron dextran accentuates the toxicity of N. meningitidis and soman reduced the toxicity in the presence and absence of iron. These results support the previous data that soman produced an immunosuppressive response.

All of the above results suggests that the immunosuppressive effects of soman were due to an elevation of the circulating corticosterone.

However in adrenalectomized mice soman still produced an immunosuppressive response (60% inhibition in the SRBC plaque forming assay). This suggests that some other mechanism was responsible for the immunosuppression. There is a possibility that the hypothermia after soman may play a role in the immunosuppressive response. Further experiments are contemplated to determine the exact mechanism of immunosuppression following soman poisoning.

If these results can be duplicated in higher species, it would suggest that survivors of organophosphate poisoning may be more susceptible than normal to infection and should be treated accordingly in the clinical situation, especially in the situation where combined casualties are evident, i.e. those having open wounds in addition to nerve agent poisoning. These combined casualties present a special problem for the physician. There are a number of areas where drug interactions could have dire consequences (i.e. anesthetics, muscle relaxants, etc). The problem of drug interactions in combined casualties is an area of research which has been largely ignored to date but which should be actively persued.

Effect of Nerve Agent Poisoning on Thyroid Hormones

The secretions of the thyroid gland, thyroxine (T4) and triiodothyronine (T3) are extremely important hormones in maintenance of metabolic homeostasis (Table 8). The effects of soman poisoning on T3 and T4 serum concentrations measured by RIA (Endocrin Sci, Tarzana, Ca.) were examined. Soman was injected at 1000 hrs and samples taken at 1300, 1600 and 1900 hrs of the same day and at 0800 of the following day. The results in Table 9 demonstrate that serum T3 concentrations were increased significantly ($p<0.01$) at all time periods following soman (287 μg/kg) and at 1600 and 1900 hrs following soman (100 μg/kg). Following soman poisoning (100 and 287 μg/kg) T4 concentrations (Table 10) were elevated to a greater extent and

were significantly different ($p<0.01$) from controls at 1300, 1600 and 1900 hrs. Again these effects following poisoning were not long-lasting. By 0800 hrs of the day following poisoning both T3 and T4 levels were not significantly different from control. However Rumsey et al. (1981) reported that long-term exposure to Ronnel, a systemic insecticide and antihelminthic, resulted in elevated T3 and T4 levels in cattle. T4 stimulated hepatic drug metabolism of androgen-dependent substrates such as ethylmorphine (Colby and Rumbough, 1985). The increased serum concentration of thyroid hormones could contribute to the increased ethylmorphine N-demethylase activity (Table 6) following soman poisoning. The nature of the increase in serum T3 and T4 is unknown as TSH levels were not measured. However, it is highly probable that the increase in T3 and T4 following soman poisoning is the result of a decreased metabolic breakdown due to the intense hypothermia rather than an increased secretion of hormones by the thyroid although a direct inhibitory effect on the steroid hydroxylating enzymes cannot be ruled out (Donovan et al., 1978).

Effect of Nerve Agent Poisoning on Testosterone

Testosterone produced by testis and adrenal cortex plays an important role in sexual development in immature mammals (i.e. secondary sex characteristics). In adults its primary role is to maintain the physiological state of reproductive accessory ducts and glands and possesses an anabolic action. Serum testosterone concentrations, measured by RIA (Amersham International), following soman poisoning (100 and 287 μg/kg, sc) were significantly lower ($p<0.01$) at all time periods examined (Table 11). However, by 0800 hrs next day the testosterone concentrations were back to control levels. In rodents the scrotal temperature is 4-7°C lower than body temperature which at the deepest part of the hypothermia following soman poisoning would put the temperature of the scrotum in the range of 22-25°C. At this point the production and release of testosterone may be severely

reduced. Alternatively the stress response could mediate the decrease in testosterone levels (Opstad and Aakvaag, 1983). Since the reduction of testosterone is only transient it is unlikely that any long-term effect on spermatogenesis and thus fertility would result.

Effects of Organophosphate Poisoning
Possibly Mediated by Hormones

Various effects noted following organophosphate poisoning could be mediated by hormonal changes (Table 12). For example, hyperglycemia could result from the generalized stress response, i.e. adrenaline release from adrenal medulla stimulates glycogenolysis. This combined with increased corticosterone concentrations, which decrease glucose utilization peripherally, could result in hyperglycemia. In an earlier study in rats it was found that soman poisoning did not have any significant effect on plasma insulin levels (Clement and Lee, 1980). A similar interaction of adrenaline and corticosterone could increase lipolysis leading to the hyperlipemia noted following organophosphate poisoning (Fukuyama et al., 1963).

Increases in cerebellum c-GMP have been noted following soman poisoning (Lundy and Magor, 1978; Lundy and Shaw, 1983). Stress (cold water swimming and immoblization) also produced similar increases in c-GMP in cerebellum (Dinnendahl and Gumulka, 1977). It is possible that the increased levels of c-GMP following soman could be the result of a generalized stress reaction and not necessarily a direct effect of the organophosphate.

Various investigators have noted overshoots in recovery of plasma cholinesterase activity after organophosphate poisoning, biphasic time-course of recovery of acetylcholinesterase and a stimulation of protein synthesis in liver (see Table 12). These effects could be the result of increased corticosterone concentrations stimulating protein synthesis although a direct

effect on protein synthesis cannot be ruled out (Cisson and Wilson, 1981). The decrease in RNA after organophosphate poisoning could also be due to glucocorticoid action (see Table 3). RNA levels in control animals appear to be under the influence of a diurnal rhythm (Anthony et al., 1983) similar to that of glucocorticoids.

CONCLUSIONS

The results of these studies show that organophosphate poisoning upsets homeostasis. Coincident with this, hormonal changes may affect a wide variety of systems *in vivo*. The entire picture of organophosphate poisoning in survivors is extremely complex. Biochemical and/or neurochemical changes observed may be the normal response to an alteration or reseting of homeostasis at another level rather than a direct effect of organophosphate poisoning *per se*. These factors should be considered when attempting to interpret the nature and/or significance of *in vivo* data. In light of the intense hypothermia after nerve agent poisoning in rodents it would appear to be extremely important to utilize appropriate controls. If biochemical and/or neurochemical measurements (neurotransmitters, hormones, etc) are made within the first 4 hrs of poisoning it would be prudent to include a control group which was also hypothermic to differentiate the soman responses from the hypothermic responses. Perhaps an appropriate dose of oxotremorine or maybe nicotine would suffice since it produces similar cholinergic crisis and time-course of hypothermia in the absence of cholinesterase inhibition. The problem of interpretation and extrapolation is made even more difficult by the fact that not all of the "nerve agents" produce the same responses even though their primary mechanism of action is similar, i.e. inhibition of acetylcholinesterase. Species differences in response to nerve agent poisoning further complicate the problem.

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TABLE 1

Other Effects of Organophosphate Poisoning

Area	Reference
1. GABAergic	Sivam et al., 1983 Coudray-Lucas et al., 1984 Lundy et al., 1978 Sivam et al., 1984
2. Cyclic nucleotides	
a) C-AMP	Dinnendahl and Stock, 1975 Sevaljevic et al., 1981 Sevaljevic et al., 1984 Grisk et al., 1980
b) C-GMP	Lundy and Magor, 1978 Lundy and Shaw, 1983
3. Catecholamines	Coudray-Lucas et al., 1983 Prioux-Guyonneau et al., 1982
4. Behavioral Effects	Beleslin, 1981 Kux Minskaia et al., 1984 Landauer, 1984 D'Mello, 1983 Hoskins et al, 1983

TABLE 1 (Cont'd)

Other Effects of Organophosphate Poisoning

Area	Reference
5. Analgesia	Clement and Copeman, 1983 Zadik et al., 1983 Koehn et al., 1979
6. Neuropathology	Petras, 1981 McLeod et al., 1984 Lemercier et al., 1983 Samson et al., 1984
7. Delayed Neuropathy	Abou-Donia, 1981 Johnson, 1981 Johnson, 1980 Gordon et al., 1983
8. Cardiac Effects	Mederos, 1982 Kiss and Fazekas, 1983 Kiss and Fazekas, 1979 Clay and Stewart, 1982 Hirshberg and Lerman, 1984
9. Tolerance	Oiianas et al., 1984 Yamada et al., 1983 Costa et al., 1982 Churchill et al., 1984

TABLE 1 (Cont'd)

Other Effects of Organophosphate Poisoning

Area	Reference
	Schwab et al., 1983 Dulaney et al., 1983
10. Myopathy	Dettbarn, 1984
11. Receptor-Ionic Channel Complex Interactions	Albuquerque et al., 1984 Karczmar and Ohta, 1981
12. Depot	Van Helden and Wolthuis, 1983 Van Helden et al., 1984 Clement, 1982 Sterri et al., 1982
13. Detoxification (Aliesterase or Phosphorylphosphatase)	Clement, 1984 Sterri et al., 1981 Nordgren et al., 1984 Hoskin et al., 1984 Benschop et al., 1984 Losch et al., 1982
14. Anticonvulsants	Lundy and Shaw, 1983 Stroykov, 1979 Vinogradov, 1971 Lipp, 1973

TABLE 2

Inhibition of Various Enzymes Other Than Cholinesterase by Organophosphate Anticholinesterases

In Vivo	
Succinate dehydrogenase	Jovic et al., 1971
$Na^{+}K^{+}$-ATPase	Jovic et al., 1971
Ca^{++}-ATPase	Dierkes-Tizek et al., 1984
aldolase	Jovic et al., 1971
tyrosine hydroxylase	Richardson et al., 1976
aliesterase	Clement, 1984
acylcoenzyme A: cholesterol acyltransferase	Civen et al., 1980
neurotoxic esterase	Abou-Donia, 1981
triglyceride lipase	Dinnendahl et al., 1973
In Vitro	
chymotrypsin	Ooms and Van Dijk, 1966
trypsin	Ooms and Van Dijk, 1966
phosvitin kinase	Hokin et al., 1966
adenylate cyclase	Grisk et al., 1980
protein kinase	Grisk et al., 1980
lecithin-cholesterol acyltransferase	Nakagawa et al., 1982
diglyceride kinase	Hokin et al., 1966
prolyl endopeptidase	Kato et al., 1980
rat liver kininase	Mazzacoratti and Sampaio, 1982
elastase	Naughton et al., 1960
phosphoglucomutase	Milstein and Sanger, 1961
subtilisin	Sanger and Shaw, 1960

TABLE 2 (Cont'd)

Inhibition of Various Enzymes Other Than Cholinesterase
by Organophosphate Anticholinesterases

alkaline phosphatase	Milstein, 1963
lipoprotein lipase	Korn and Quigley, 1957
acid phosphatase	Greenberg and Bachmansohn, 1962
testosterone hydroxylase	Donovan et al., 1978
c-AMP phosphodiesterase	Civen et al., 1980
sterol-ester hydrolase	Civen et al., 1977a

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TABLE 3

Actions of Glucocorticoids

Area	Action	Reference
Carbohydrate Metabolism	Stimulate gluconeogenesis and decrease glucose utilization. Glucocorticoids decrease amino acid transport intracellularly in extrahepatic cells and combined with continued protein catabolism plasma amino acid levels increase which stimulate gluconeogenesis in liver (i.e. conversion of amino acids into glucose). Induction of hepatic enzymes involved in gluconeogenesis (phosphoenolpyruvate carboxykinase, fructose-1,6-diphosphatase and glucose-6-phosphatase)	Thomas & Bell, 1982 Haynes & Murad, 1980
Protein Metabolism	Stimulate transcription and synthesis of specific proteins such as tyrosine aminotransaminase and tryptophan pyrrolase. A reduction in cellular protein content in extrahepatic tissue whereas in liver production of cellular protein and plasma proteins is increased. Increases quantity of mRNA that codes for enzymes whose synthesis is stimulated by glucocorticoids. There is evidence that glucocorticoids stimulate the synthesis of an inhibitory protein which causes catabolic effects (e.g. lymphoid cells and fibrinoblasts).	Nelson, 1983 Thomas & Bell, 1982 Schutz et al., 1975 Iynedjian & Hanson, 1977 Makman et al., 1971
Fat Metabolism	Mobilize fatty acids from fat depots resulting in increased plasma fatty acid levels (plasma lipids are not changed consistently in hypo or hypercorticism). Combined with increased fatty acid oxidation is responsible for conversion of glucose to fatty acid as energy source during stressful periods.	Thomas & Bell, 1982 Haynes & Murad, 1980

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TABLE 3 (Cont'd)

Actions of Glucocorticoids

Area	Action	Reference
Anti-Inflammatory Response and Immune System	Suppress phospholipase A_2 activity and liberation of arachadonic acid thus decreasing formation of prostaglandins, prostacyclin and thromboxane. Possess lympholytic properties and can alter phagocytic properties of macrophages although they do not produce any effect on proliferating stem cells of bone marrow. However, lymphocytopenia thought to be result of redistribution rather than destruction. Decreases collagen formation thus limiting wound healing. Decreases the mass of lymphoid tissue. Inhibits thymocyte cell growth and/or division. Suggestion that this is a steroid/receptor action which induces synthesis of protein which inhibits various cellular processes. Glucocorticoids don't have an effect on antibody titers. In delayed sensitivity reactions glucocorticoids block the effect of macrophage inhibitory factor and thus non-sensitized machrophages do not accumulate locally. Glucocorticoids induce production of a factor which stimulates migration of ppolymorpho-nuclear leucocytes.	Wolfe, 1982 Haynes & Murad, 1980 Luster et al., 1982 Dougherty & White, 1947 Balow & Rosenthal, 1973 Stevenson, 1973 Sharma, 1981 Fauci & Dale, 1974 Pohorecky & Wurtman, 1971 Vermes et al., 1976
Catecholamine Synthesis	Glucocorticoids influence epinephrine biosynthesis in adrenal medulla by controlling phenylethanolamine N-methyltransferase activity. Play a modulatory role in maintaining functional activity of certain central serotinergic neurons.	

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TABLE 3 (Cont'd)

Actions of Glucocorticoids

Area	Action	Reference
Behavioral Effects	Influence mood, behavior, EEG and brain excitability perhaps directly through specific receptors although some changes can be ascribed to electrolyte inbalance. Increase perception of various senses such as taste, smell and sound.	Ballard et al., 1974 Henkin, 1970
Electrolytes	Cortisol is of major importance in maintaining a difference in the Na^+ and K^+ concentrations of the intra- and extracellular compartments. In rodents corticosterone (the major glucocorticoid) differs from cortisol in that it has mineralo- and glucocorticoid actions.	Nelson, 1983 Haynes & Murad, 1980
Neurotransmission	Increase β adrenoceptors in rat lung and decrease β receptors in liver. Improve neuromuscular function in myasthenia gravis and antagonize the neuromuscular blocking action of hemicholinium-3. Reduce duration of ketamine and pentobarbitone-induced anesthesia.	Salonen & Mattila, 1984 Wolfe et al., 1976 Seybold & Drackman, 1974 Wolters et al., 1974 Leeuwin et al., 1984

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TABLE 4

Effect of Soman Poisoning on
Serum Corticosterone Levels in Mice

Time of day (hr)	Corticosterone (μg/100 ml)		
	Soman (μg/kg)		
	0	100	287[a]
1000	5.4 ± 0.3[b]	5.4 ± 0.3	5.4 ± 0.3
1300	8.5 ± 0.4	20.4 ± 1.5[c]	23.0 ± 0.8[c]
1600	8.5 ± 0.9	18.1 ± 1.3[c]	22.5 ± 1.1[c]
1900	11.0 ± 0.3	12.9 ± 1.3	21.7 ± 2.3[c]

[a] mice received atropine (17.4 mg/kg) and HI-6 (50 mg/kg) ip 5 min before soman sc

[b] mean ± SEM (N = 8-10 observations)

[c] significantly different from control ($p < 0.01$)

TABLE 5

Effect of Soman Poisoning on
Plasma ACTH

Time of day (hr)	ACTH levels (pg/ml) Soman (μg/kg) 0	100	287[a]
1000	99.3 ± 6.8[b]	99.3 ± 6.8	99.3 ± 6.8
1300	102.2 ± 10.3	69.2 ± 5.7[c]	113.5 ± 13.4
1600	106.0 ± 7.3	79.7 ± 9.2	99.5 ± 11.2
1900	96.0 ± 4.6	109.3 ± 9.1	95.5 ± 6.9

[a] mice received atropine (17.4 mg/kg) and HI-6 (50 mg/kg) ip 5 min before soman sc

[b] mean ± SEM (N = 8-18 observations)

[c] significantly different from control ($p < 0.05$)

TABLE 6

Effect of Soman Poisoning on the Activity of Hepatic Mixed Function Oxidase Activity[a]

Time After Soman (hr)	Soman (100 μg/kg)			Soman (287 μg/kg)		
	Aniline Hydroxylase	Ethylmorphine N-demethylase	p-nitroanisole O-demethylase	Aniline Hydroxylase	Ethylmorphine N-demethylase	p-nitroanisole O-demethylase
2	99	165[d]	93	99	155[d]	94
24	97	130[d]	102	96	128[c]	102
48	-	143[d]	121	-	148[d]	121

[a] Specific activity for various mixed function oxidases: aniline hydroxylase, 0.68 μg p-aminophenol/mg protein/15 min; ethylmorphine N-demethylase, 9.3 μg formaldehyde/mg protein/10 min; p-nitroanisole o-demethylase, 38.2 μmoles p-nitrophenol/mg protein/20 min.

[b] % control activity

significantly different from control [c]($p<0.05$); [d]($p<0.001$)

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TABLE 7

Effect of Soman Poisoning on the "In Vivo" Toxicity of Neisseria meningitidis in Mice[a]

Soman	Iron Dextran	No. Mice Used	No. Mice Dead	% Mortality
-	-	10	1	10
-	+	10	9	90
+	-	10	0	0
+	+	10	5	50

[a] All mice were injected (ip) with Neisseria mengingitidis. Soman (100 µg/kg; sc) was administered at least 2 hrs prior to injection of the bacteria. Iron dextran (250 mg/kg; ip) was injected immediately after the bacteria. Mortality values were obtained 72 hrs after bacteria injection.

TABLE 8

Cellular Effects of Thyroid Hormones

Increase cell turnover and development
Increase protein anabolism and catabolism
Increase lipid turnover
Increase oxygen consumption and heat production
Increase sensitivity of myocardium to catecholamines (hyperthyroidism)
Regulates hepatic microsomal drug metabolism

TABLE 9

Effect of Soman Poisoning on
Serum Triiodothyronine (T3)

Time of day (hr)	T3 (μg/100 ml) Soman (μg/kg) 0	100	287
1000	95.9 ± 4.9[a]	95.9 ± 4.9	95.9 ± 4.9
1300	92.3 ± 7.2	97.1 ± 4.6	123.9 ± 4.8[b]
1600	91.2 ± 6.1	115.3 ± 4.6[b]	116.6 ± 2.8[b]
1900	86.4 ± 5.0	102.2 ± 3.4[b]	112.5 ± 2.6[b]

[a] mean ± SEM (N = 17-20 observations)

[b] significantly different from control ($p < 0.01$)

TABLE 10

Effect of Soman Poisoning on
Serum Thyroxine (T4)

Time of day (hr)	T4 (μg/100 ml) Soman (μg/kg) 0	100	287
1000	10.9 ± 0.6[a]	10.9 ± 0.6	10.9 ± 0.6
1300	11.3 ± 0.8	25.5 ± 1.8[b]	29.4 ± 2.9[b]
1600	12.1 ± 0.5	26.6 ± 1.9[b]	26.9 ± 3.0[b]
1900	13.2 ± 0.8	23.5 ± 1.9[b]	32.4 ± 1.4[b]

[a] mean ± SEM (N = 8-10 observations)

[b] significantly different from control ($p < 0.01$)

TABLE 11

Effect of Soman Poisoning on
Serum Testosterone

Time of day (hr)	Testosterone (ng/ml)		
	Soman (μg/kg)		
	0	100	287
1000	8.6 ± 1.6[a]	-	-
1300	-	-	-
1600	6.1 ± 1.9	0.8 ± 0.1[b]	0.7 ± 0.1[b]
1900	6.9 ± 1.1	1.4 ± 0.4[b]	2.4 ± 1.0[b]

[a] mean ± SEM (N = 8-10 observations)

[b] significantly different from control ($p < 0.01$)

TABLE 12

Effects Following Organophosphate Poisoning
Which May be Mediated by Hormones

Hyperglycemia	Matin & Siddiqui, 1982 Moore & James, 1981 Meller et al., 1981 Lober et al., 1984
Hyperlipidemia	Fukuyama et al., 1963
Increase in c-GMP	Lundy & Magor, 1978 Lundy & Shaw, 1983
Stimulation of protein synthesis	Domschke et al., 1970a Domschke et al., 1970b Swisher et al., 1984 Stitcher et al., 1977
Overshoot in cholinesterase activity after poisoning	Grubic et al., 1981 Glow et al., 1966 Locker & Siedek, 1951 Frawley et al., 1952
Biphasic recovery of acetylcholinesterase after poisoning	Grubic et al., 1981 Clement (unpublished observations)
Decrease in RNA (brain)	Doebler et al., 1983 Anthony et al., 1983

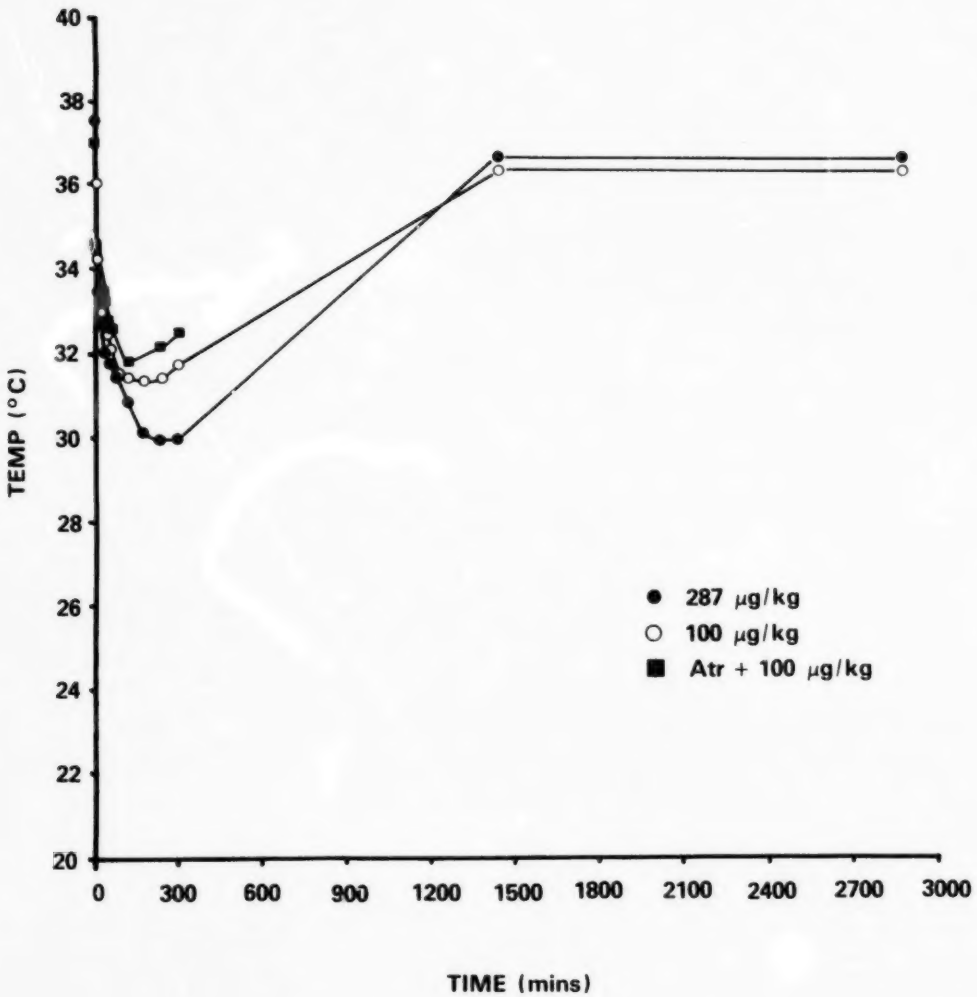

Figure 1

Time Course of Soman-induced Hypothermia in Mice

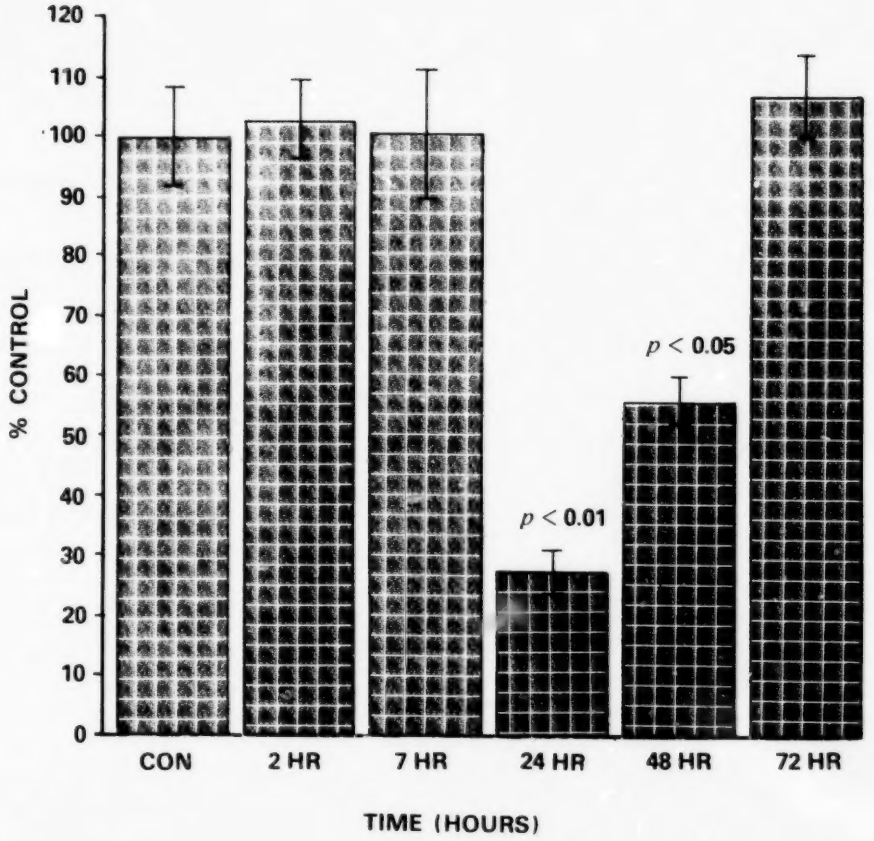

Figure 2

Time Course of the immunosuppressive effect of Soman. Mice were injected with SRBC at "0" time. at various times after, Soman (100 μg/kg; sc) was administered. Four days after SRBC injection the mice were sacrificed and a standard plaque assay (Jerne *et al.*, 1963) performed.

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Hormonal changes were estimated after poisoning with an extremely toxic organophosphate, soman (pinacolyl methylphosphonofluoridate). Soman produced a significant ($p<0.05$) increase in serum corticosterone, thyroxine and triiodothyronine concentrations at 3, 6 and 9 hrs after poisoning. However, after 22 hrs the levels were not significantly different from control. Plasma ACTH levels were decreased significantly ($p<0.05$) 3 hrs after soman at 100 μg/kg but not after 287 μg/kg. Serum testosterone levels were decreased significantly ($p<0.01$) 6 and 9 hrs after soman poisoning but had returned to control levels by 22 hrs.

Soman (100 μg/kg) produced an immunosuppressive response when administered at 24 hrs after an antigen (sheep red blood cells). However a similar response was obtained in adrenalectomized mice suggesting that some mechanism other than elevated corticosterone was responsible for the immunosuppressive effect.

Soman poisoning produced an intense hypothermia. It is possible that the hormonal changes noted after soman poisoning are due to a reduction in metabolism and excretion, a result of the hypothermia and not a direct action of soman.

KEY WORDS

soman

sarin

tabun

immunotoxicology

hormones

ACTH

corticosterone

thyroxine

triidothyroxine

testosterone

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